

Characterisation of neonicotinoid resistance in the cotton aphid, *Aphis gossypii* from Australian cotton

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By

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(Hons)**

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Certificate of Original Authorship

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Contents

Certificate of Original Authorship	i
Refereed Journal Publications	ii
Conference Proceedings.....	iii
Acknowledgements	iv
Contents	v
List of Tables.....	ix
List of Figures	xi
List of Abbreviations and Acronyms	xvi
Abstract	xix
Chapter 1. Review of literature	1
1.1 The cotton aphid, <i>Aphis gossypii</i>	1
1.1.1 Life cycle of <i>Aphis gossypii</i>	3
1.1.2 Economic damage caused by <i>Aphis gossypii</i>	5
1.1.3 Management of <i>Aphis gossypii</i>	6
1.1.4 History of insecticide resistance in <i>Aphis gossypii</i>	10
1.2 The Neonicotinoids	12
1.2.1 Development and Structure	13
1.2.2 Agricultural and economic importance	15
1.2.3 Target site	16
1.2.4 Selectivity of neonicotinoid insecticides towards insect nAChRs	18
1.3 Insecticide Resistance Mechanisms	20
1.3.1 Target site insensitivity.....	21
1.3.2 Metabolic detoxification.....	26
1.4 Techniques available for resistance detection	33
1.4.1 Bioassay	33
1.4.2 Bioassay with synergist.....	35

1.4.3 DNA sequencing	36
1.4.4 Next generation sequencing	37
Aims, objectives and thesis format.....	40
Chapter 2. Characterisation and maintenance of three thiamethoxam resistant strains of the cotton aphid <i>Aphis gossypii</i> for use in subsequent experimental chapters	42
2.1 Abstract.....	42
2.2 Introduction	42
2.3 Methods and Materials.....	43
2.3.1 Collection and maintenance of strains	43
2.3.2 Plant germination and strain culturing	44
2.3.3 Discriminating dose tests	45
2.3.4 Pressuring	45
2.3.5 Full log-dose probit tests	46
2.3.6 PCR screening of two known mutations: S431F, associated with pirimicarb (carbamate) resistance; and L1014F, associated with pyrethroid resistance.....	46
2.4 Results	48
2.4.1 Discriminating dose tests	48
2.4.2 PCR restriction enzyme assays	49
2.4.3 Full log-dose probit tests	49
2.5 Discussion.....	54
Chapter 3. Efficacy of two thiamethoxam pre-germination seed treatments and a phorate side-dressing against neonicotinoid and pirimicarb resistant cotton aphid <i>Aphis gossypii</i>	57
3.1 Foreword.....	57
3.2 Abstract.....	58
3.3 Introduction	58
3.4 Materials & Methods	60
3.4.1 Chemicals tested.....	60

3.4.2 Aphids	60
3.4.3 Thiamethoxam trial.....	60
3.4.4 Phorate trial.....	61
3.4.5 Statistical analysis.....	61
3.5 Results	62
3.5.1 Thiamethoxam trial.....	62
3.5.2 Phorate trial.....	70
3.6 Discussion.....	76
Chapter 4. Resistance mechanisms associated with the neonicotinoid insecticide thiamethoxam in Australian pest populations of the cotton aphid <i>Aphis gossypii</i>	78
4.1 Foreword.....	78
4.2 Abstract.....	78
4.3 Introduction	78
4.4 Methods and Materials.....	80
4.4.1 Aphids	80
4.4.2 Chemicals	81
4.4.3 Non-synergist and synergist bioassays.....	81
4.4.4 Data Analysis	81
4.4.5 Primer Design	82
4.4.6 RNA Extraction and cDNA synthesis	82
4.4.7 PCR amplification of R81T mutation site.....	83
4.5 Results.....	83
4.5.1 Dose responses with and without synergist	83
4.5.2 PCR amplification of complementary DNA containing the R81T mutation site	84
4.6 Discussion.....	86
Chapter 5. Characterisation of the cotton aphid <i>Aphis gossypii</i> transcriptome under thiamethoxam stress identifies transcripts associated with insecticide resistance.....	89

5.1 Abstract.....	89
5.2 Introduction	89
5.3 Methods and Materials.....	91
5.3.1 Aphids	91
5.3.2 <i>Aphis gossypii</i> cDNA library construction and sequencing.....	92
5.3.3 Assembly and functional annotation.....	93
5.3.4 Analysis of transcript expression differences between resistant and susceptible transcriptomes.....	93
5.3.5 Quantitative RT-PCR	93
5.4 Results.....	94
5.4.1 Illumina sequencing and sequence assembly.....	94
5.4.2 Gene ontology (GO) and Clusters of orthologous groups (COGs) classification.....	95
5.4.3 Network of unigene	100
5.4.4 Differential expression and pathway analyses in resistant vs susceptible strain combinations	100
5.4.5 Candidate resistance (detoxification) genes	100
5.4.6 Quantitative RT-PCR	101
5.5 Discussion.....	102
Chapter 6. General discussion	106
6.1 Future work.....	112
Appendix A. Supplementary material referred to in all Chapters.....	114
Appendix B. Primers used in this study	115
Appendix C. Supplementary material from Chapter 3.....	117
Appendix D. Supplementary material from Chapter 4.....	124
Appendix E. Supplementary material from Chapter 5.....	130
Bibliography	157

List of Tables

Table 1.1 Insecticides registered for control of <i>Aphis gossypii</i> in Australian cotton as arranged by their corresponding MoA group (CottonInfo 2015, IRAC 2016).....	9
Table 1.2 Insecticides documented worldwide to which <i>Aphis gossypii</i> has developed resistance as a result of field exposure or laboratory selection (Whalon et al. 2008)..	11
Table 1.3 List of neonicotinoids and related compounds registered for use in Australian cotton for the control of sucking insect pests (Maas 2014).	16
Table 2.1 Resistance detection (percent susceptible) in <i>Aphis gossypii</i> strains Sus SB, F 101, Glen tw n S and Carr using bioassay [Thia (thiamethoxam) and Clo (clothianidin)] and molecular [Pir (pirimicarb) and Pyr (pyrethroid)] based methodology.	49
Table 2.2 Full log dose response data for the reference susceptible <i>Aphis gossypii</i> strain Sus SB against formulated thiamethoxam (Actara [®] 250 g/kg).....	50
Table 3.1 Wald-F test statistics for fixed effects of thiamethoxam analysis.	64
Table 3.2 Non-zero variance component and standard error (SE) for random terms of thiamethoxam analysis.....	65
Table 3.3 Estimated treatment efficacies (Et) and approximate 95% confidence intervals (CI) of two varying rates of formulated thiamethoxam (g a.i./kg seed) against neonicotinoid susceptible and neonicotinoid resistant <i>Aphis gossypii</i>	66
Table 3.4 Wald-F test statistics of fixed effects for phorate analysis.	71
Table 3.5 Non-zero variance component and standard error (SE) for random terms of phorate analysis.....	72
Table 3.6 Estimated treatment efficacies (Et) and approximate 95% confidence intervals (CI) of phorate as a side dressing against pirimicarb susceptible and pirimicarb resistant <i>Aphis gossypii</i>	73

Table 4.1 Probit mortality data for thiamethoxam + PBO against <i>Aphis gossypii</i> strains susceptible F 96 and resistant pressured F 101 _P , Glen twn S _P and Carr _P	84
Table 5.1 Summary of reads and assembly from Illumina sequencing for <i>Aphis gossypii</i> strains: reference susceptible Sus F 96 and thiamethoxam resistant F 101, Glen twn S and Carr.....	95
Table A.1 Discriminating dose data of <i>Aphis gossypii</i> thiamethoxam resistant strains (F 101, Glen twn S and Carr) after routine pressuring with varying rates of thiamethoxam (Actara [®] 250 g/kg).	114
Table B.1 Primers used in Chapter 2.	115
Table B.2 Primers used in Chapter 4.	115
Table B.3 Primers used in Chapter 5.	116
Table E.1 Summary of KEGG pathway mapping of ‘A_Gossypii_Aust-Unigene’.	137
Table E.2 Top differentially expressed genes (DEGs) among thiamethoxam resistant (F 101, Glen twn S and Carr) and reference susceptible (Sus F 96) <i>Aphis gossypii</i> strains. Transcript ID, log ₂ FC, p-value and orthologue gene name in the appropriate organism as retrieved from GenBank (Blast nr) are indicated. False discovery rate≤0.05 and p-value≤0.001 were thresholds for determining the significance of gene expression differences.....	148
Table E.3 Four differentially expressed transcripts by qRT-PCR analysis among thiamethoxam resistant (F 101, Glen twn S and Carr) <i>Aphis gossypii</i> strains (compared to the reference susceptible Sus F 96).....	156

List of Figures

Figure 1.1 Colour polymorphism of adult <i>Aphis gossypii</i> . A, dwarf yellow apterae; B, light green apterae; C, dark green apterae; D, winged (alate) adult.	1
Figure 1.2 Life cycle of aphids (Shingleton et al. 2003).....	3
Figure 1.3 The structure of the synthetic insecticide imidacloprid. Also shown are other synthetic insecticides that are related to imidacloprid: nithiazin, nitenpyram, acetamiprid, dinotefuran, clothianidin and thiamethoxam. The two main moieties of the imidacloprid molecule are shown; the tertiary amine that corresponds to the quaternary ammonium of ACh and the nitro group of imidacloprid are highlighted in red and blue, respectively. Substitution at the 1-position of nithiazin led to the eventual production of imidacloprid, based on which further neonicotinoids have been synthesised (Matsuda et al. 2001).	14
Figure 1.4 A schematic representation of the arrangement of a heteromeric acetylcholine receptor consisting of two α (dark grey) and three non- α (light grey) subunits arranged around a central cation-permeable channel. Acetylcholine binding sites: ACh; Four transmembrane domains: 1-4; Six binding loops: A-F; Cys-loop: two white circles connected by a white double line (Jones and Sattelle 2010).....	17
Figure 1.5 Graphic representation of the types of genetic mutations which occur and cause resistance. (a) the gene is amplified to produce more copies of itself and thus increase the amount of gene product made (b) the regulatory expression of a gene is modified so that the amount of gene product made is increased (c) modification of the gene sequence produces a structurally different product (Scott 1995).....	21
Figure 2.1 Location of aphid collections: A, Moree; B, Toobeah; C, St George.	44
Figure 2.2 Dose–response for <i>Aphis gossypii</i> against thiamethoxam (F 101) and following three years of continual laboratory selection and maintenance (F 101 _p) (Susceptible SB has been redrawn from Table 2.2 to add clarity).	51

Figure 2.3 Dose–response for <i>Aphis gossypii</i> against thiamethoxam (Glen twn S) and following three years of continual laboratory selection and maintenance (Glen twn S _P) (Susceptible SB has been redrawn from Table 2.2 to add clarity).....	52
Figure 2.4 Dose–response for <i>Aphis gossypii</i> against thiamethoxam (Carr) and following three years of continual laboratory selection and maintenance (Carr _P) (Susceptible SB has been redrawn from Table 2.2 to add clarity).	53
Figure 3.1 Fitted trend for the thiamethoxam analysis, for each strain by treatment combination (thiamethoxam at 5.52 g a.i./kg seed, Cruiser Extreme [®] Insecticide Seed Treatment; thiamethoxam at 2.76 g a.i./kg seed, Cruiser [®] Insecticide Seed Treatment; untreated cotton seed, variety Sicot 71). The solid line represents the fitted trend, with dotted lines representing the 95% confidence interval. The raw data for each replicate is numbered 1 to 3 in each panel (with replicates 1 and 3 shifted slightly left and right, respectively, to avoid overlap).	69
Figure 3.2 Fitted trends for the phorate analysis, for each strain by treatment combination (phorate equivalent to 3 kg/ha, Thimet [®] 200 G Systemic Granular Insecticide; untreated cotton seed, variety Sicot 71). The solid line represents the fitted trend, with dotted lines representing the 95% confidence interval. The raw data for each replicate is numbered 1 to 3 in each panel (with replicates 1 and 3 shifted slightly left and right respectively, to avoid overlap).	75
Figure 4.1 Amino acid alignment of a partial sequence of nAChR β 1 subunit from <i>Aphis gossypii</i> strains Sus F 96, F 101, Glen twn S, Carr and IMI-R (Imidacloprid resistant, GenBank accession number: AFH00994.1), including partial nAChR β 1 subunit gene sequences from two related aphid species <i>Aphis glycines</i> (GenBank accession number: JN681174.1) and <i>Myzus persicae</i> (GenBank accession number: AJ251838.1) resulting from the ClustalW method. A conserved loop (Loop D) within the ligand binding domain is marked by a red box. A known point mutation site (R81T) in the loop D region of the β 1 subunit is marked in bold.....	85

Figure 5.1 Pie-charts showing distributions from BLASTx matches of pooled *Aphis gossypii* transcriptome unigenes with respect to (A) E-values (B) gene identity and (C) insect species from which the homologous genes were matched to. 96

Figure 5.2 Clusters of Orthologous Groups (COG) of protein function classification of *Aphis gossypii* unigene sequences (a total of 7633 unigenes were grouped into COG function classifications). A: RNA processing and modification, B: Chromatin structure and dynamics, C: Energy production and conversion, D: Cell cycle control, cell division, chromosome partitioning, E: Amino acid transport and metabolism, F: Nucleotide transport and metabolism, G: Carbohydrate transport and metabolism, H: Coenzyme transport and metabolism, I: Lipid transport and metabolism, J: Translation, ribosomal structure and biogenesis, K: Transcription, L: Replication, recombination and repair, M: Cell wall/membrane/envelope biogenesis, N: Cell motility, O: Posttranslational modification, protein turnover, chaperones, P: Inorganic ion transport and metabolism, Q: Secondary metabolites biosynthesis, transport and catabolism, R: General function prediction only, S: Function unknown, T: Signal transduction mechanisms, U: Intracellular trafficking, secretion, and vesicular transport, V: Defence mechanisms, W: Extracellular structures, Y: Nuclear structure, Z: Cytoskeleton. 97

Figure 5.3 GO annotations of all combined unigenes and DEG sequences. GO categories shown in the x axis are grouped into three main ontologies: biological process, cellular component and molecular function. The right y-axis indicates the number of genes in each category, while the left y-axis indicates the percentage of total genes in that category. The ‘A_Gossypii_Aust_unigene’ indicates that the unigenes were those assembled from reads from the pooled transcriptome of all strains. 99

Figure 5.4 Validation of gene expression of four transcripts selected from RNA-Seq analysis. (a) The fold change (\log_2 Ratio) for genes from RNA-Seq analysis between strain comparisons: F 101/Sus F 96; Glen twn S/Sus F 96; and Carr/Sus F 96 (b) The relative expression of four transcripts between strain comparisons: F 101/Sus F 96;

Glen twn S/Sus F 96; and Carr/Sus F 96, calculated by qRT-PCR using comparative threshold cycle method.	101
Figure D.1 Primer-BLAST results based on the nicotinic receptor $\beta 1$ subunit of <i>Aphis gossypii</i> (GenBank accession number JQ627836.1) used to design primers in Table B.2.	127
Figure E.1 Schematic diagram illustrating the experimental pipeline of transcriptome assembly used in this study.	130
Figure E.2 Length distribution of contigs. 'A_Gossypii_Aust-Contig' indicates that the contigs were those assembled from reads from the pooled transcriptome of four <i>Aphis gossypii</i> strains [including the reference susceptible (Sus F 96) and thiamethoxam resistant (F 101, Glen twn S and Carr)].	131
Figure E.3 Length distribution of unigenes. 'A_Gossypii_Aust_Unigene' indicates that the unigenes were those assembled from reads from the pooled transcriptomes of four <i>Aphis gossypii</i> strains [including the reference susceptible (Sus F 96) and thiamethoxam resistant (F 101, Glen twn S and Carr)].	131
Figure E.4 Histogram presentation of the gene ontology classification. GO categories, shown in the x-axis, are grouped into three main ontologies: biological process, cellular component and molecular function. The right y-axis indicates the number of genes in each category, while the left y-axis indicates the percentage of total genes in that category. The 'F96-VS-F 101' indicates that the unigenes were those assembled from reads from the comparison of a reference susceptible (Sus F 96) and thiamethoxam resistant (F 101) <i>Aphis gossypii</i> strains.	132
Figure E.5 Histogram presentation of the gene ontology classification. GO categories, shown in the x-axis, are grouped into three main ontologies: biological process, cellular component and molecular function. The right y-axis indicates the number of genes in each category, while the left y-axis indicates the percentage of total genes in that category. The 'F96-VS-Glen' indicates that the unigenes were those assembled	

from reads from the comparison of a reference susceptible (Sus F 96) and thiamethoxam resistant (Glen twn S) *Aphis gossypii* strains. 133

Figure E.6 Histogram presentation of the gene ontology classification. GO categories, shown in the x-axis, are grouped into three main ontologies: biological process, cellular component and molecular function. The right y-axis indicates the number of genes in each category, while the left y-axis indicates the percentage of total genes in that category. The 'F96-VS-Carr' indicates that the unigenes were those assembled from reads from the comparison of a reference susceptible (Sus F 96) and thiamethoxam resistant (Carr) *Aphis gossypii* strains. 134

Figure E.7 PRALINE alignment of the predicted cytochrome P450 gene 6k1-like of the pea aphid *Acyrtosiphon pisum* (Accession number: XP001948421.1) and *Aphis gossypii* sequence Contig 1190 (firstly translated using ExPASy (Gasteiger et al. 2003)). 135

Figure E.8 PRALINE alignment of the predicted cytochrome P450 gene 6k1-like of the pea aphid *Acyrtosiphon pisum* (Accession number: XP001948421.1) and *Aphis gossypii* sequence Contig 1418 (firstly translated using ExPASy (Gasteiger et al. 2003)). 136

List of Abbreviations and Acronyms

ABC: Adenosine triphosphate-binding cassette

***Ace*:** Acetylcholinesterase gene

AChE: Acetylcholinesterase (the target of organophosphate and carbamate insecticides)

ACh: Acetylcholine

ATP: Adenosine triphosphate

CBT: Cotton Bunchy Top

CE: Carboxylesterase

CLR: Cotton Leaf Roll

COG: Cluster of orthologous groups

cys-LGIC: cys-loop Ligand gated ion channel

CYP: Family of P450 genes

DEF: *S,S,S*-tributyl phosphorotrithioate (a synergist)

ddNTPs: di-deoxynucleotidetriphosphates

DDT: Dichlorodiphenyltrichloroethane

DEG: Differentially expressed genes

EMAI: Elizabeth Macarthur Agricultural Institute

EMS: Ethyl methanesulfonate

EST: Esterase

FPKM: Fragments aligned per thousand bases per million reads

GABA: gamma-aminobutyric acid (the target receptor of organochlorines and Phenylpyrazoles (Fiproles) insecticides)

GST: Glutathione-S-transferase

GO: Gene Ontology

IPM: Integrated Pest Management

IRAC: Insecticide Resistance Action Committee

IRMS: Insecticide Resistance Management Strategy

***kdr*; super-*kdr*:** knock down resistance (knock-down resistance traits)

KEGG: Kyoto Encyclopedia of Genes and Genomes

LC₅₀: The dose required to kill half the tested population

LC₉₉: The dose required to kill 99% of the tested population

LBD: Ligand binding domain

nAChR: Nicotinic acetylcholine receptor (the target of neonicotinoid insecticides)

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NHC: Nitromethylene heterocyclic compounds

NGS: Next generation sequencing

NR: Non-redundant

NSW: New South Wales

***o-Ace*:** orthologous gene to *Drosophila* gene *Ace*

***p-Ace*:** paralogous gene to *Drosophila* gene *Ace*

PBO: Piperonyl butoxide (a detoxification enzyme inhibitor)

PCR: Polymerase chain reaction

P450: Cytochrome P450-dependent monooxygenase

QLD: Queensland

qRT-PCR: Quantitative real-time polymerase chain reaction

***Rdl*:** Resistance to dieldrin gene

RR: Resistance ratio

RFLP: Restriction fragment length polymorphism

RNA-Seq: RNA-Sequencing

SNP: Single nucleotide polymorphism

UGT: UDP-glucuronosyltransferase

USA: United States of America

USD: United States dollar

VGSC: *para*-type voltage gated sodium channel (the target of pyrethroid insecticides, pyrethrins and DDT)

WHO: World Health Organisation

Abstract

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a highly polyphagous pest that inflicts serious damage to a broad range of agricultural, horticultural and greenhouse crops. In Australia, *A. gossypii* is a significant pest of cotton and is difficult to control with insecticides because of its high propensity to develop resistance. Neonicotinoids are among the most effective insecticides used to control *A. gossypii* but the recent detection of resistance threatens their longevity. Consequently, I aimed to restore neonicotinoid efficacy against *A. gossypii* through elucidation of underlying resistance mechanism(s).

Bioassay was used to measure thiamethoxam (neonicotinoid) response in three field strains collected from commercial cotton. Resistance ratios between 49- and 85-fold were produced and resistance was correlated with potential field control failures via a glasshouse efficacy trial. Results showed that resistant *A. gossypii* could complete their development on cotton grown from thiamethoxam-treated seed. A second trial investigated the use of phorate (an organophosphate) as an alternative pre-germination treatment to thiamethoxam. Phorate effectively controls neonicotinoid resistant *A. gossypii* but cross resistance between phorate and the carbamate insecticide pirimicarb must be carefully considered as part of any sustainable management strategy.

PCR-Sequencing was employed to identify if mutation R81T known to confer resistance to neonicotinoid compounds was present in Australian *A. gossypii*. Comparative sequence analysis between susceptible and resistant strains confirmed the absence of mutation R81T. Potential biochemical mechanisms of thiamethoxam resistance in *A. gossypii* were then studied using synergist bioassays. The use of the synergist piperonyl butoxide in tandem with thiamethoxam completely or partially suppressed resistance. This suggests that resistance is at least in part, mediated by overexpression of detoxification enzymes that could subsequently be targeted to achieve improved field control of resistant aphids.

High-throughput sequencing of the *A. gossypii* transcriptome found differences in gene expression associated with thiamethoxam resistance. Two transcripts involved in the

detoxification of xenobiotics (putatively annotated as cytochrome P450 gene *6K1*-like) were found differentially expressed between resistant and susceptible strains. Transcript expression was further validated by qRT-PCR and showed a similar tendency in up-regulation of expression. As such I identified this gene as the strongest candidate for thiamethoxam resistant *A. gossypii*.

This study has generated a comprehensive transcriptome resource for *A. gossypii* that has characterised the expression of numerous important transcripts encoding proteins involved in insecticide resistance. Consequently, my study will contribute to future research relating to molecular characterization of insecticide resistance mechanisms in *A. gossypii* and other insect pests.